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Vaccine efficacy of porcine reproductive and respiratory syndrome virus chimeras

Joshua S. Ellingson[a], Yue Wang[b], Sarah Layton[c], Janice Ciacci-Zanella[d,1], Michael B. Roof[c], Kay S. Faaberg[b,d,*]

[a] Department of Veterinary Microbiology and Preventative Medicine, Iowa State University, Ames, IA 50011, USA
[b] Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN 55108, USA
[c] Boehringer Ingelheim Vetmedica, Inc., Ames, IA 50010, USA
[d] Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, Ames, IA 50010, USA

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ABSTRACT

The vaccine efficacy of six PRRSV Type 2 infectious clones, including five chimeras and a strain-specific deletion mutant, were examined using a respiratory challenge model in growing swine. The chimeras were constructed from different combinations of a licensed modified live vaccine (Ingelvac® PRRS MLV) and a virulent field isolate (*wt* MN184) which differ by 14.3% on a nucleotide basis, while the deletion mutant tested had a broad deletion in the nsp2 region of strain MN184. The appearance of antibodies and virus characterization revealed regions of the genome that could influence PRRSV replication *in vivo*. Swine growth, clinical signs and lung lesions were also monitored. Average daily weight gain was negatively and directly impacted by some vaccines, and after challenge, vaccination with different constructs led to variable weight gain. We determined that 3 of the tested chimeras, including two previously published chimeras [1] and one in which strain MN184 ORF5-6 was placed on the background of Ingelvac® PRRS MLV were able to prevent lung consolidation to a similar extent as traditionally prepared cell-passaged attenuated vaccines. The study suggested that only specific chimeras can attenuate clinical signs in swine and that attenuation cannot be directly linked to primary virus replication. Additionally, the strain MN184 deletion mutant was not found to have been sufficiently attenuated nor efficacious against heterologous challenge with strain JA-142.

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1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) emerged to cause clinical problems in animals in the early 1990s on separate continents [2,3]. Since then, the virus has spread to most swine producing regions of the world. PRRSV has been found to vary as much as 40% in nucleotide sequence and has been separated into two genotypes, European (Type 1) and North American (Type 2), based upon their original isolation location and date. As a result of this overwhelming diversity, the swine immune response is often not cross protective. PRRSV also induces a poor immune response in most animals. This incomplete protection appears to be due to several factors including the nature of the virus, the genetics of swine host and the complication of co-infection with other pathogens [4,5]. One traditional method of vaccine preparation, culturing the virus over several *in vitro* cell passages in order to attenuate clinical symptoms, has resulted in several available products for use in the field [6,7]. However, this can lead to incomplete protection against heterologous PRRSV strains and an uncertainty in selection of the appropriate vaccine for routine use [5]. A newer approach has been to evaluate infectious cDNA clones of PRRSV, which can represent chimeras or site-specific changes that may potentially increase the immune response, and may also have specifically engineered deletions and/or insertions to provide markers for vaccine identification [1,8–16].

Limited reports using this new approach have suggested that specific PRRSV chimeras can provide direct attenuation of clinical signs in either a respiratory model or a reproductive failure model [1,10]. Our previous studies have demonstrated that two reciprocal chimeras (rMLVORF1/MN184 and rMN184ORF1/MLV) of Type 2 PRRSV strains, Ingelvac® PRRS MLV and *wild-type* (*wt*) isolate MN184, could attenuate clinical signs of young swine after heterologous challenge with PRRSV strain SDSU73 in a respiratory challenge model [1]. Therefore, it was hypothesized that additional chimeras created from the same parent strains, but with different

* Corresponding author at: USDA-ARS, Virus and Prion Research Unit, National Animal Disease Center, 1920 Dayton Avenue, P.O. Box 70, Mail Stop 2S-209 Ames, IA 50010, USA. Tel.: +1 515 663 7259; fax: +1 515 663 7458.
E-mail address: kay.faaberg@ars.usda.gov (K.S. Faaberg).
[1] Permanent address: Embrapa Suínos e Aves, Concórdia, Santa Catarina, Brazil.

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regions of the genome exchanged, would be more efficacious in the face of virulent challenge than commercially available products and perhaps provide an alternate method of PRRSV attenuation.

To extend these original studies, we examined the two original [1] as well as three additional chimeras, plus a deletion mutant recombinant of *wt* strain MN184, under different challenge conditions. The new genomic areas of study were chosen to consider the contributions of key regions of a virulent strain in inducing protection against subsequent heterotypic virus exposure. PRRSV ORF5-6 code for the major viral attachment domain, ORF7 encodes the nucleocapsid protein that surrounds the genome and interacts with the structural proteins in the virion, and the 3′UTR is critical to successful transcription of subgenomic RNAs and replication [17,18]. The additional chimeras were synthesized using an Ingelvac® PRRS MLV backbone. In addition, the replicase region known as nonstructural protein 2 (nsp2) has been shown to be immunogenic, contains hypervariable segments, encodes a protease responsible for replicase cleavage and harbors B-cell epitopes [12,19–25]. Thus, in order to examine a possible role for nsp2 in protection, recombinant strain MN184 was modified by removing 618 bases of the nsp2 coding region. The six engineered viruses were used as vaccines in parallel with two conventionally attenuated PRRSV strains, Ingelvac® PRRS MLV and newly prepared MN184 (MN184-P102). The vaccinated animals were then challenged with *wt* Type 2 strain JA-142. The appearance of antibodies and virus characterization were followed over the course of the study. The results of these assays revealed regions of the genome that influence PRRSV replication *in vivo*. Swine growth, clinical signs and lung lesions were also monitored. Average daily weight gain was negatively and directly impacted by some vaccines, and after challenge, vaccination with different constructs led to variable weight gain. We also found that only the original chimeras and the one in which strain MN184 ORF5-6 was placed on the background of Ingelvac® PRRS MLV were able to prevent lung consolidation after strain JA142 challenge to a similar extent as the cell-passaged attenuated vaccines. The outcomes of this study suggested that only specific chimeras can attenuate clinical signs in swine and that attenuation cannot be directly linked to primary virus replication. Additionally, a large deletion in the nsp2 region of strain MN184 was not sufficient to reduce the pathogenicity of that strain, or serve as an adequate vaccine against heterologous challenge with strain JA-142.

2. Materials and methods

2.1. Cells and viruses

MA-104 cells (ATCC CRL2621) or MARC-145 cells, both African Green monkey kidney cell lines which support the growth of PRRSV, were cultured in minimum essential medium (EMEM, SAFC Biosciences M56416) with 10% fetal bovine serum (Invitrogen) at 37 °C, 5% CO_2. PRRSV vaccine Ingelvac® PRRS MLV and *wt* isolate MN184 were previously described [1,26]. Two recombinant viruses, rMLV and rMN184, and two chimeric viruses, rMLVORF1/MN184 and rMN184ORF1/MLV, were rescued from cDNA clones (GenBank EF484031–EF484034) described previously [1]. Other recombinant viruses were generated as described below. MN184-P102 was prepared by successive passages of MN184C (GenBank EF488739) on MARC-145 cells at Boehringer Ingelheim Vetmedica, Incorporated. PRRSV strain JA-142 (AY424271) was used as a heterologous challenge virus for the swine studies and has also been characterized previously [27,28].

2.2. Construction of PRRSV cDNA clones

Different sections of pMLV were replaced with comparable sections of pMN184 using specific restriction enzyme sites (Fig. 1) or the primers listed in Table 1. The correct nucleotides of the exchanged regions of every clone were confirmed by DNA sequencing. The nucleotide and amino acid changes as a result of the cloning are listed in Table 2.

pMLV/MN184ORF5-6 (GenBank Accession FJ629369) possessed nucleotides (nt) 1–13,650 and 14,823–15,452 of pMLV; nt 13,651–14,822 were exchanged for pMN184 13,257–14,429 by PciI and SmaI restriction digest of subclone IV of each full-length plasmid (Fig. 1). pMLV/MN184ORF7-3′UTR (GenBank Accession FJ629370) consisted of nt 1–14,822 of pMLV; nt 14,823–15,452 were replaced by pMN184 14,430–15,060 by SmaI digestion of subclone IV of each full-length plasmid. In both constructs, the new subclone IV replaced its counterpart in pMLV. The specific regions targeted span nucleotides 13,789–14,391 (ORF5), 14,376–14,900 (ORF6) and 14,890–15,452 (ORF7 and 3′UTR) of the parental virus, Ingelvac® PRRS MLV.

pMLV/MN184-3′UTR (GenBank Accession FJ629371) was obtained in the following manner. One PCR product was ampli-

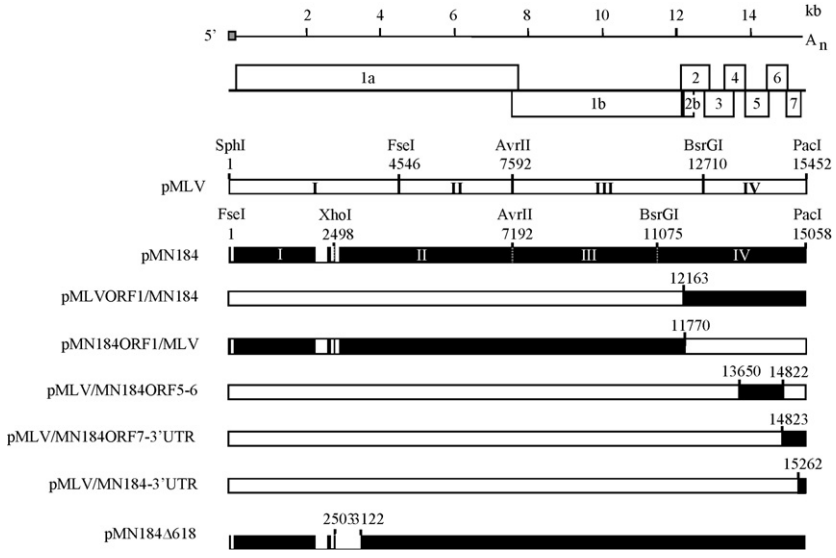

Fig. 1. Genome schematic of PRRSV, the two infectious clones initially derived (pMLV and pMN184 [1]) and chimeric and deletion mutants prepared from these clones for the present study.

Table 1
Primers used in preparation of recombinant PRRSV infectious clones. Forward primers indicated by a slash (/) following the name and reverse primers by a slash before the name. Primers were positioned based on genomic sequences pMLV (MLV; EF484033) or pMN184 (MN184; EF484031) (most primers anneal to both sequences).

Primer	Nucleotide Position	Sequence
Synthesis of PRRSV recombinants		
MLV-ORF6-F/	MLV 14,192–14,217	5′-GCTACGCGTGTACCAGATATACCAAC
/MLV-ORF7-R	MLV 15,249–15,277	5′-CAAGAATGCCAGCTCATCATGCTGAGGGT
184-3′UTR-F/	MN184 14,856–14,884	5′-ACCCTCAGCATGATGAGCTGGCATTCTTG
/184-3′UTR-R	MN184 15,011–15,099	5′-GTCTTTAATTAACTAG$(T)_{30}$AATTTCGGC
184-3122-F/	MN184 2494–2503/3122–3136	5′-AAGCTCGAGCTGTGGGTTTGTGATG
/184-4083-R	MN184 4065–4091	5′-AAAACCGGTCGCACAGGTCGACAAGTG

Table 2
Nucleotide and amino acid changes to recombinant viral parent due to clone construction.

Construct	Region	NT Changes	AA Changes
pMLV/MN184ORF5-6	GP4	11	0
	GP5	81	31
	M	37	6
pMLV/rMN184ORF7-3′UTR	M	5	2
	N	26	6
	3′UTR	9	–
pMLV/MN1843′UTR	3′UTR	9	–
pMN184Δ618	nsp2	Δ618	Δ206

fied using pMLV and primer pair MLV-ORF6-F/MLV-ORF7-R and another product representing the 3′UTR of MN184 was amplified from pMN184 using 184-3′UTR-F/184-3′UTR-R. Overlapping PCR was then completed with both PCR products and MLV-ORF6-F/184-3′UTR-R. The PCR product was then digested with SmaI and PacI and cloned into subclone IV of pMLV, which was then used to replace part IV in pMLV. The final pMLV/MN1843′UTR construct possessed nucleotides nt 1–15,261 of MLV with only the 3′UTR (15,262–15,452) replaced with 14,869–15,058 of pMN184.

As reported, *wt* strain MN184 has a tripartite deletion totaling 393 bases in the nsp2 region when compared to other sequenced viruses [20]. It was of interest to assess additional nucleotide deletions in regards to MN184 strain virulence reduction and the capacity of the mutated virus to protect against strain JA-142 challenge. One PCR product was amplified using pMN184 and primer pair 184-3122-F/184-4083-R, which was then digested with XhoI and AgeI, cloned into subclone II of pMN184 and then into a full-length viral plasmid. As a result, a 618 nucleotide segment of nonstructural protein 2 (nt 2504–3121) was removed from pMN184 to produce the final pMN184Δ618 construct (14, 440 bp; GenBank Accession FJ629372).

2.3. Rescue of viruses

The cDNA clones were linearized with PacI and then transcribed *in vitro* (mMessage Machine Kit, Applied Biosystems). RNA transcripts (2.5 μg) of each clone were subsequently transfected into confluent MA-104 cells using DMRIE-C (Invitrogen), as described previously [1]. The transfection supernatants were collected when cytopathic effect (CPE) was approximately 80% and cell debris was then removed by centrifugation at $4000 \times g$. Recombinant viruses were passaged on MA-104 cells a total of 4 times to yield viral stocks sufficient in volume and titer to allow for vaccination studies. The rescued viruses were named rMLV/MN184ORF5-6, rMLV/MN184ORF7-3′UTR, rMLV/MN184-3′UTR and rMN184Δ618. Total RNA was extracted from an aliquot of passage 4 supernatant of each rescued virus and analyzed by RT-PCR followed by 3′ end sequencing of approximately 4000 bases.

2.4. Swine study

The study utilized 100, healthy 3-week old, commercial crossbred piglets from a PRRSV seronegative herd to examine PRRSV vaccine efficacy in a respiratory challenge model. Animals were housed in a conventional setting at Veterinary Resources Inc. in Ames, Iowa and were under the supervision of a veterinarian. Throughout the duration of the study, all animals received food and water *ad libitum*. All laboratory personnel and animal caretakers involved with the study were blinded to the treatments given to the respective groups.

The study consisted of 10 groups, including 6 infectious clones (groups 1–6), *wt* MN184 at passage 102 (MN184-P102; group 7), Ingelvac® PRRS MLV (group 8), heterologous challenge virus *wt* JA-142 (group 9), and a strict control group (group 10) (Table 3). Animals were required to test negative for PRRSV antibody by HerdChek® PRRS ELISA 2XR and then randomly assigned by weight into each treatment group prior to vaccination (IDEXX Laboratories Inc., Westbrook, ME). Viral titers were determined by $TCID_{50}$/ml (Table 3) [29,30]. All viruses were diluted with minimum essential medium (MEM; Sigma, St. Louis, MO) containing 2% fetal bovine serum (FBS) (Sigma, St. Louis, MO) in order to deliver 4.79 logs of virus in 2 ml, intramuscularly, to each animal. The challenge control (group 9) and strict control (group 10) groups received only dilution medium. For testing purposes, 10–15 ml of blood was collected from each animal on Days 0, 3, 7, 14, 21, 28, 31, and 35. Serum was separated from the clotted blood and stored for a maximum of 24 h at 4 °C for testing. Aliquots were then frozen at −70 °C.

Table 3
Treatment list, titer information and whole genome percent nucleotide identity to the challenge virus.

Group #	Treatment	Original titer (Log_{10} $TCID_{50}$/ml)	Percent nucleotide identity to JA-142
1	rMN184ORF1/MLV	5.02	84.2
2	rMLVORF1/MN184	5.70	90.2
3	rMLV/MN184ORF5-6	5.63	90.7
4	rMLV/MN184ORF7-3′UTR	5.50	90.9
5	rMN184Δ618	5.35	90.9
6	rMLV/MN184-3′UTR	4.96	80.4
7	MN184-P102	4.49	83.1
8	Ingelvac® PRRS MLV	4.09	91.0
9	N/A		
10	N/A		

The challenge model used in this study is consistent with the model used in the original characterization of the chimeras [1]. This model calls for virulent challenge three weeks after vaccination (Day 21) and necropsy at five weeks post-vaccination (Day 35). Challenge timing was chosen since the vaccine component of the chimeras, Ingelvac® PRRS MLV, has proven to be efficacious three weeks post-vaccination even though the vaccine virus may still be causing some viremia [31]. Therefore, it was expected that the chimeras would exhibit similar characteristics. Necropsy at two weeks post challenge allowed for maximal detection of lung lesions that correlate with *in vitro* testing procedures [32]. The animals in groups 1–9 were challenged intranasally with 3.8 logs in 2 ml (1 ml per nostril) of virulent JA-142 strain of PRRSV at cell passage 4. At necropsy all animals were humanely euthanized and assessed for gross lung lesions.

2.5. Clinical evaluation

General observations of each animal in the study were taken from Day 0 through Day 19 and anything abnormal was noted. From Day 20 through Day 35, clinical observations were noted and anything considered abnormal was recorded. Individual observations consisting of behavior, respiration, and cough were also recorded based on a numerical index from 1 to 4 that reflected the severity of the diseased state for each category. For instance, a normal animal received a score of 3 (3×1 each for behavior, respiration and cough), an animal exhibiting maximum clinical signs received a 9 (3×3), and a deceased animal received a cumulative score of 12 (3×4). In addition, animals were weighed 3 days before vaccination (Day −3), at challenge (Day 21), and at necropsy (Day 35) for average daily weight gain testing. The lungs of all animals in the study were evaluated at necropsy for percent consolidation due to PRRSV infection. Lungs were scored for each individual lobe, as well as an overall level of gross lung pathology using a standard scoring system [33]. The observation score equaled the sum of all the individual lobe scores.

2.6. Serology

Serum samples were analyzed for PRRSV antibody using the IDEXX HerdCheck® PRRS ELISA 2XR. The tests were performed as described by the manufacturer's instructions. Samples were considered positive for PRRSV antibodies if the sample-to-positive (S/P) ratio was at least 0.4.

2.7. Viremia detection by virus isolation and quantitative RT-PCR

To qualitatively determine viremia, virus isolation was performed on all serum samples from all collection days. Each animal was tested by inoculating 100 μl of serum individually onto 3-day-old MA-104 cells in a 48-well tissue culture plate which was then evaluated 8 days later for signs of cytopathic effect. The percent of positive animals at each bleed date was then recorded. To attain a relative quantity of viral RNA present, quantitative RT-PCR (qRT-PCR) was also performed on all serum samples. The QIAamp® Virus BioRobot® MDx Kit was used in conjunction with the BioRobot Universal System from Qiagen (Qiagen Inc., Valencia, CA) to extract the viral RNA from the serum per manufacturers recommendations. To detect US PRRSV nucleic acid, the North American Tetracore qRT-PCR kit (Tetracore, Inc., Rockville, MD) was used as described previously [29].

2.8. Nucleotide sequence analysis

Viral RNA was extracted from serum samples from each animal at day 21. To obtain a consensus nucleotide sequence of the structural genes at this time point, RNA extracts were pooled for each group and submitted for nucleotide sequence determination (oligonucleotide primers available on request). Sequences were analyzed using Geneious Pro Version 4.7.5 (Biomatters Limited). Approximately 4000 bases were sequenced at the 3′end of the viral genome. In the case of the nsp2 deletion mutant, rMN184Δ618, a 500 base section spanning the deletion site was examined.

2.9. Statistics/biometrics

All data were imported into SAS version 9.1 for management and preliminary analysis. Data listings and summary statistics by treatment group including mean, median, standard deviation, standard error, range, 95 percent confidence limits, coefficient of variation, and frequency distributions were generated for all variables where appropriate. All parameters were compared among groups 1–9 and pair wise between groups 1–9. Group 10 (strict controls) was not included in the analyses other than summary statistics. In compliance with the methods recommended by the United States Department of Agriculture Animal Plant Health Inspection Agency, only two-sided results were reported and all comparisons were at $\alpha = 0.05$. All data were transferred to Prism 4 (Graphpad Software, Inc.) for additional statistical analyses and optimal formatting prior to publication. Virus isolation data used Fisher's exact test to determine the number of animals positive/negative per group ratio. Weights and average daily weight gain (ADWG) were tested by two-way analysis of variance (ANOVA). Lung scores for each group were analyzed by one-way ANOVA and Bonferroni's Multiple Comparison Test (95% confidence interval).

3. Results

3.1. Recovery of viruses

Two chimeric viruses, rMLVORF1/MN184 and pMN184ORF1/MLV, were previously generated [1]. Four other recombinant PRRSV full-length cDNA clones, pMLV/MN184ORF5-6, pMLV/rMN184ORF7-3′UTR, pMLV/MN184-3′UTR and pMN184Δ618 were constructed in a similar manner (Fig. 1). To verify whether these four additional cDNA clones were infectious, linearized pMLV/MN184ORF5-6, pMLV/rMN184ORF7-3′UTR, pMLV/MN184-3′UTR and rMN184Δ618 were transcribed *in vitro* and the synthetic RNAs were subsequently transfected into MA-104 cells. Day 3 post-transfection, all four transfections resulted in the appearance of CPE, indicating that the genetic exchange between the two different strains and the 618 base deletion in the nsp2 region of MN184 did not have a severe effect on the *in vitro* growth properties of the recombinants. Sequence analyses of around 4000 bases at the 3′-end of the genome confirmed that these four viruses were recovered from the respective recombinant PRRSV with no or a few scattered changes (data not shown). All chimeras were passaged 4 times on MA-104 cells in parallel with parental rMLV and rMN184 as well as Ingelvac® PRRS MLV vaccine and *wt* MN184. At each passage, onset of CPE in rMLV/MN184ORF5-6 and rMLV/MN184-3′UTR infected MA-104 cells was similar to those infected with rMLV, but appeared 1 day later for rMLV/MN184ORF7-3′UTR infected cells. CPE for rMN184Δ618 infected cells was similar to rMN184 at all four passages. Passage 4 viruses were titered and used to infect 10 animals/group in the vaccination study (Table 3).

3.2. HerdChek ELISA

After vaccination, all animals in Groups 1, 2, 3, 5, 7, and 8 tested positive by IDEXX PRRS ELISA prior to strain JA-142 virulent challenge (Day 21). Group 6 (rMN184Δ618) had 9 positive animals

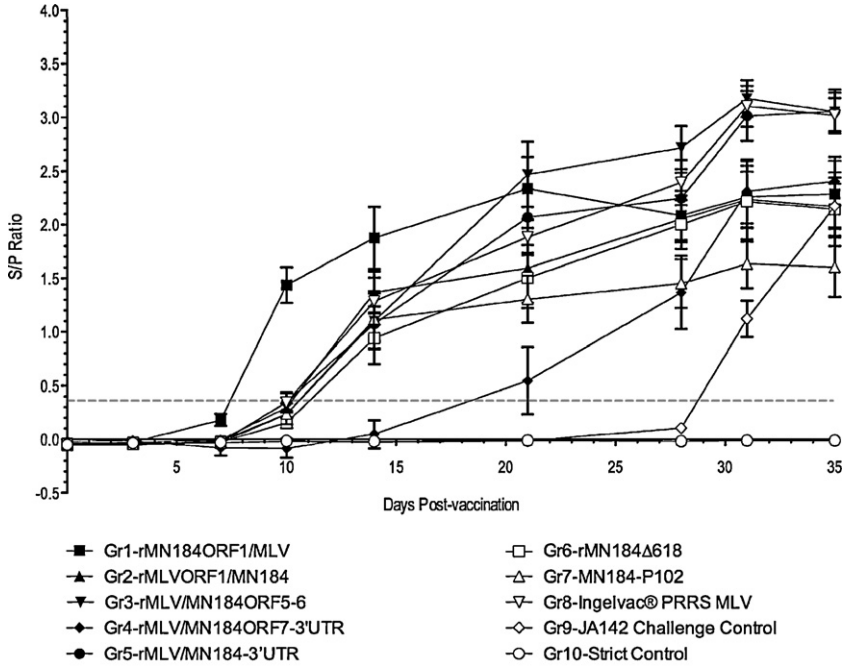

Fig. 2. Mean PRRS ELISA 2XR S/P ratios. The dashed line at 0.4 S/P ratio designates threshold value above which titers are considered positive for anti-PRRSV antibodies.

prior to challenge while Group 4 (rMLV/MN184ORF7-3′UTR) had only 4 positive animals and the appearance of the antibodies in this latter group was delayed (Fig. 2 and data not shown). Antibodies appeared in Group 1 animals 3 days prior to all other vaccination groups. In addition, the S/P ratios suggested that all of the animals in groups 1–9 seroconverted to either their respective vaccine or the challenge material by Day 31 of the study. The Strict Control (group 10) had no positive tests throughout the duration of the study. Fig. 2 indicates the day when the individual groups became positive for PRRSV specific antibodies and the trend for all treatment groups.

3.3. Virus Isolation

Virus isolation analysis confirmed that at least 3 of the 10 animals in each treatment group were viremic by Day 3 in groups 1, 2, 3, 5, 7, and 8 (Fig. 3). Only (10%) of the animals in Group 4 were positive on Days 3 and 14, the only positive results obtained for rMN184ORF7-3′UTR prior to challenge. No viremia was detected for Group 6 (rMN184Δ618) animals until Days 14 and 21, which then showed positive results for only 10% and 20% of the animals, respectively. The results suggested that each engineered recombinant virus was capable of some level of viral replication in the swine host, although it is evident that rMN184ORF7-3′UTR (Group 4) and rMN184Δ618 (Group 6) were less successful at replicating inside the animal host as compared to the other groups, as measured by virus isolation on MARC-145 cells. We had detected antibodies to Group 6 virus (Fig. 2) with similar kinetics to all other treatment groups (except Group 4) that might be indicative of replication of this virus in the absence of overt CPE due to infection of MA-104 cells. PRRSV vaccine strains MN184-P102 and Ingelvac® PRRS MLV confirmed viral replication within the host, although replication of Ingelvac® PRRS MLV from swine serum samples on cultured cells was more apparent than replication of MN184-P102 at all time points. Viremia continued after virulent heterologous PRRSV challenge in all groups except the strict control.

In order to assess the ability of the immune response to reduce the replication of the JA-142 challenge virus after vaccination with each of the candidate viruses, we compared the levels of viremia for all treatment groups to that of the Challenge Control Group on Day 35, when presumably most virus remaining in the animals would

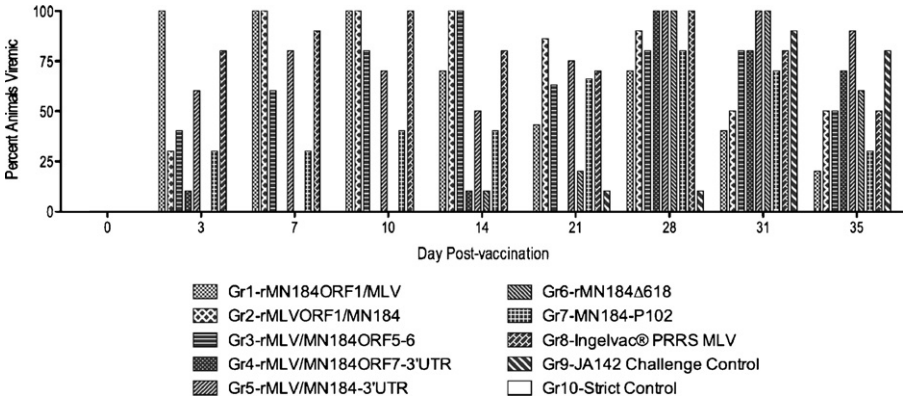

Fig. 3. Viral load, determined by virus isolation on MA-104 cells, in swine serum at all time points after intramuscular inoculation with 4.79 logs of each virus. The challenge control and strict control groups received only dilution medium. On Day 21, all animals except the strict control group were intranasally challenged with 3.8 logs of strain JA-142. Results for each animal of each group were collated and the percent positive per group was then determined.

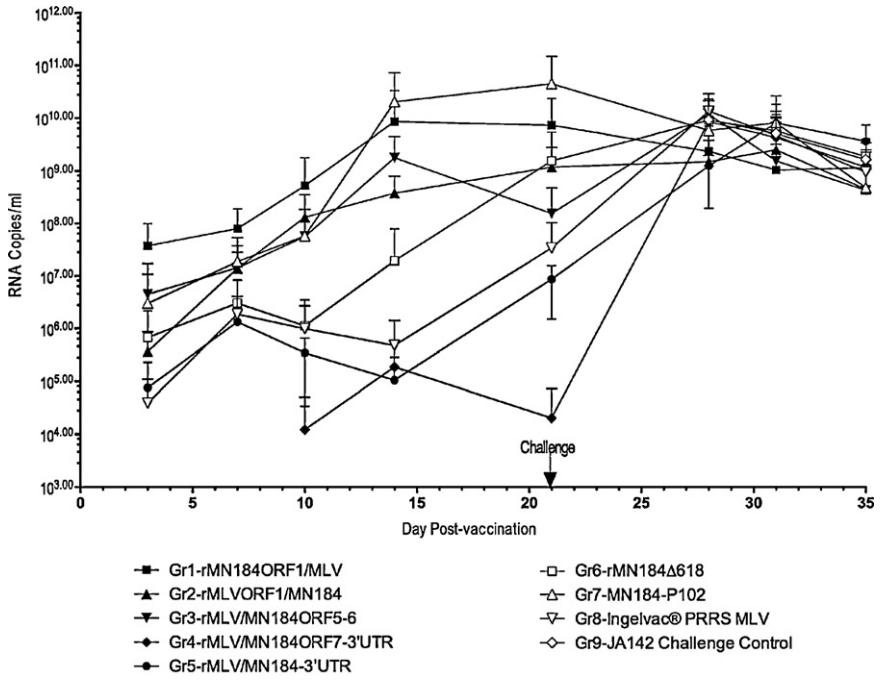

Fig. 4. Viral load was determined at all time points by qRT-PCR and plotted as viral RNA copies/ml serum. Results were plotted as the mean and error bars signify standard deviation. No viral RNA was detected in group 10 animals.

be the challenge PRRSV strain. Groups 1–3 (chimeric viruses) and Groups 7–8 (traditionally prepared vaccines) had a statistically significant ($p \leq 0.0001$) lower percentage of viremia as compared to the Challenge Control (Group 9) at this time point (Fig. 3).

3.4. Real Time RT-PCR

PRRSV RNA was detected in all pigs of treatment Groups 1–8 prior to challenge except for Group 4 (rMLV/MN184ORF7-3′UTR), for which only 3 of the 10 animals were positive for viral RNA by the day of challenge (Fig. 4). On Day 10, the various treatment viruses could be separated into two discrete categories. Those that had high levels of circulating viral RNA (>10^7) include Groups 1–3 (chimeric viruses) and Group 7 (MN184-P102) while those that had less amounts of viral RNA (<10^6) included Groups 4–6 and 8. Viral RNA detected in Group 6 animals suggested that this virus (rMN184Δ618) initially replicated at a lower rate, but eventually achieved RNA levels at Day 21 approximately equal to the viruses initially showing a higher level of circulating viral RNA. Group 4 animals revealed only 3 of 10 animals with circulating PRRSV RNA until after challenge, suggesting the virus does not replicate well in swine with a nucleocapsid gene and 3′UTR different from the rest of the pMLV genome. The remaining two viruses, inoculated into animal Groups 5 (rMLV/MN184-3′UTR) and 8 (Ingelvac® PRRS MLV), never reached above 10^7 RNA copies/ml until after challenge.

On Day 28, after virus challenge with heterologous strain JA-142, PRRSV RNA was found in all animals, providing evidence of successful challenge conditions. No treatment groups were statistically different for viral load as compared to the Challenge Control, suggesting little or no effect on JA-142 replication in swine by prior vaccination, when analyzed by qRT-PCR (Fig. 4). These results are considerably different from those obtained with virus isolation (Fig. 3).

3.5. Nucleotide sequence analysis

RT-PCR followed by nucleic acid sequencing of the products was completed on pooled and extracted serum samples from day 21. Approximately 1000 bases of ORF1b, the entire structural protein region and most of the 3′UTR were examined to ensure the animals remained infected with the respective test virus. In all cases, no discrepancy between the consensus nucleotide sequence with the input viral genome was found (data not shown). Furthermore, all viruses showed very little nucleotide variation after 21 days in 10 different animals. This indicated that all of the viruses, including the chimeras, were not undergoing demonstrable nucleotide change in the regions examined during the course of the experiment.

3.6. Average daily weight gain

To assess the gross clinical effects of PRRSV vaccination and challenge on swine, all animals were weighed at each time point. From this data, average daily weight gain (ADWG) was derived for Days −3 to 21 (before challenge) and Days 21–35 (after challenge)(Fig. 5). Prior to virulent challenge, Group 10 (Strict Control) had a statistically significant ($p \leq 0.01$) higher ADWG than only rMN184ORF1/MLV (Group 1), signifying that only Group 1 vaccination significantly reduced animal growth during the period before challenge (identified as A in Fig. 5). After challenge, Groups 1 (rMN184ORF1/MLV), 5 (rMLV/MN184-3′UTR), 6 (rMN184Δ618), and 9 (JA-142 Challenge Control) showed significantly reduced ADWG ($p \leq 0.01$) compared to control animals. This reduced ADWG may be due to insufficient protection of animals by prior vaccination in Groups 1, 5 and 6. To monitor the ability of the various viruses to protect against reduced weight gain after JA-142 challenge, ADWG was compared to Group 9 animals (Challenge Control)(identified as B in Fig. 5). In this comparison, rMLVORF1/MN184 (Group 2), rMLV/MN184ORF5-6 (Group 3), rMN184ORF7-3′UTR (Group 4), MN184-P102 (Group 7), and Ingelvac® PRRS MLV (Group 8) showed a statistically significant ($p \leq 0.05$) higher ADWG than the JA-142 Challenge Control group.

3.7. Clinical observations

Very few animals exhibited clinical signs after primary infection with the test viruses or with controls, with only one out of

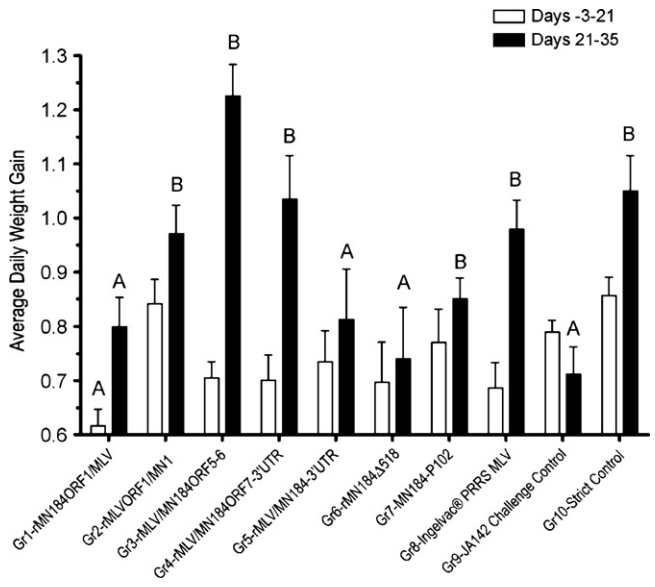

Fig. 5. Growth effects of chimeric and parental viruses on swine. All experimental pigs were weighed at Days −3, 21 and end of study. The average daily weight gain (ADWG) from 10 pigs in each group was calculated at period of −3 to 21 and 21–35 dpi. The mean was plotted and the standard error of the mean (SEM) represented as error bars. Statistically significant (≤0.01) lower average daily weight gain than the Strict Control group for the relevant time period was specified by the letter A. Statistically significant (≤0.01) higher average daily weight gain than Challenge Control group for Days 21–35 is represented by the letter B.

ten animals in each of Groups 5–7 showing mild discomfort (data not shown). This suggests that all treatments, although replicating variably in the host, did not mimic overt PRRS disease typically seen in the field. Only one animal in Group 6 experienced sustained mild lethargy and/or an intermittent cough after challenge (data not shown). In all, the mild clinical signs were to be expected and were a typical response to PRRSV infection in high health herds. The symptoms were not severe enough to have an effect on the outcome of the study, as attending veterinarians determined that no medication was necessary for resolution of clinical signs for all animals enrolled in the study.

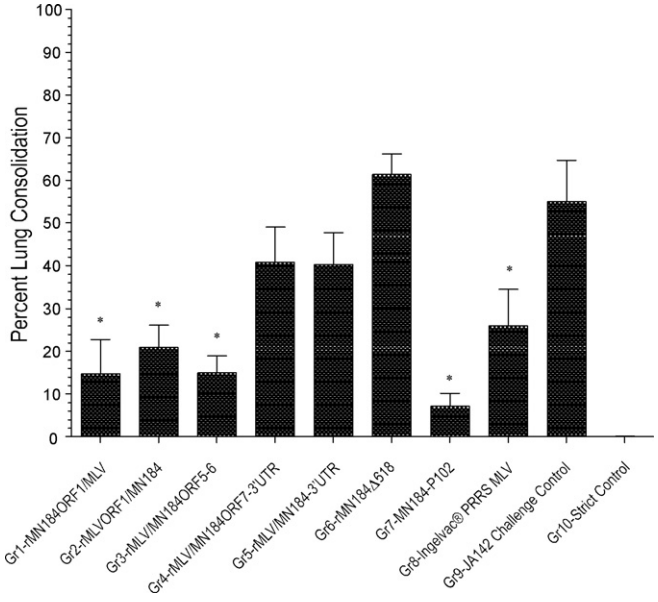

Fig. 6. Average lung scores were recorded at 35 dpi. The results were plotted as mean values of gross lung lesions from 10 pigs in each group, and the SEM values from different pigs designated by error bars. An asterisk indicates the average lung score of the group is lower than the challenge control group (*; $p < 0.01$ to $p < 0.05$).

3.8. Lung pathology

Upon completion of the study (Day 35), all animals were necropsied and assessed for lung pathology (Fig. 6). When compared to the Challenge Control (group 9), five treatment groups exhibited a statistically significant reduction in gross lung lesions. Those groups were: rMN184ORF1/MLV (Group 1; $P < 0.001$), rMLVORF1/MN184 (Group 2; $P < 0.01$), rMLV/MN184ORF5-6 (Group 3; $P < 0.001$), the recently developed vaccine MN184-P102 (Group 7; $P < 0.001$) and, to a lesser degree, Ingelvac® PRRS MLV (Group 8; $P < 0.05$). Three groups, rMN184ORF7-3′UTR (Group 4), rMLV/MN184-3′UTR (Group 5), and rMN184Δ618 (Group 6), did not appear to have sufficient protection against the development of pulmonary lesions in the strain JA-142 respiratory challenge model as the average lung scores of these three groups were not significantly different (>0.05) than the average score of the Challenge Control Group, which had over 50% of the lung displaying lesions. The Strict Control (group 10) had no lung lesions, thus indicating a valid challenge and successful bio-containment.

4. Discussion

In this report, a respiratory challenge model was used to examine the vaccine efficacy of five chimeras and a deletion mutant engineered from PRRSV Type 2 strain viral clones that differed by 14.3% on a nucleotide basis. Two chimeras, rMN184ORF1/MLV (Group 1) and rMLVORF1/MN184 (Group 2) had been previously shown to successfully protect swine against challenge with heterologous PRRSV strain SDSU73 [1]. The percent nucleotide identities between the Group 1 and 2 chimeras and SDSU73, over the available SDSU73 ORF2-7 sequence (EF442775), were 92.9% and 89.7%, respectively. For this study, the nucleotide identities based on complete genome comparisons to strain JA-142 ranged from 80.4 to 91.0% (Table 3), and yet two of the four most efficacious vaccines were of lower identity. One conclusion to draw from these comparisons is that percent similarity is not an accurate measure for determining which vaccine formula will provide the best protection from challenge, as has been shown previously for ORF5 only [34]. Rather, PRRSV protection after vaccination seems to be directed towards specific gene regions that influence genome replication kinetics and/or viral interaction with the swine host. Since both ORF1 reciprocal chimeras protected against strain SDSU73 and now strain JA-142, and both replicated well in swine, we firmly established that genome components from both viral nonstructural and structural regions can influence the ability to protect against heterologous challenge [1]. The present work also suggests that simple exchange of just the ORF5-6 region of strain MN184 can protect against challenge with strain JA-142, possibly increased over the traditionally prepared Ingelvac® PRRS MLV vaccine. This specific data reveals similar findings as those completed using a reproductive challenge model and infectious clones of two other PRRSV strains, attenuated vaccine Prime Pac PRRS® and virulent NVSL #97-7895 [10]. The rMN184 nsp2 deletion mutant (Group 6) also provided interesting results. As in previous study findings, where full-length rMN184 did not protect against challenge with strain SDSU73 [1], rMN184Δ618 did not protect against challenge with strain JA-142. The challenge viruses were different between those two studies, so additional parallel experiments must be completed to substantiate this preliminary finding. However, the data suggested that deletion of much of the nsp2 hypervariable region did not improve protection from heterologous PRRSV challenge. All of the data confirmed that PRRSV attenuation is complex, and may

involve interactions between individual viral component and/or host factors.

Novel findings concerning viral fitness *in vivo* were also presented. Virus isolation, which requires another round of MA-104 cell infection and growth, revealed that rMLV/MN184ORF7-3′UTR and rMN184Δ618 both replicated at a slower rate than most other viruses before challenge (Fig. 3). rMLV/MN184-3′UTR replicated quite well *in vitro*. However, when samples were directly assessed for the level of serum vRNA by qRT-PCR, rMLV/MN184-3′UTR along with rMLV/MN184ORF7-3′UTR may have replicated very poorly in swine, suggesting a PRRSV strain does not easily tolerate a nucleocapsid gene or protein and/or a 3′UTR different from the rest of the genome. rMN184Δ618 showed evidence of adequate replication *in vivo* when monitored by qRT-PCR, different from what was detected by the virus isolation technique. The implications of this finding are that viral fitness must be directly examined in the host, that replication of chimeric viruses in the host animal are not predictable and, therefore, one must assess several parameters when evaluating viruses for pharmaceutical use. We have also shown that some chimeric viruses can be readily utilized as vaccines, although traditionally prepared vaccines can perform as well or better against specific virulent strains.

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